Interview Summary	Application No. 10/522,405	Applicant(s) COSSARIZZA, ANDREA	
	Examiner MARK STAPLES	Art Unit 1637	

All participants (applicant, applicant's representative, PTO personnel):

(1) MARK STAPLES. (3)_____.

(2) SANDY LIVNAT. (4)_____.

Date of Interview: 06/05/09 (ended).

Type: a)☒ Telephonic b)☐ Video Conference
c)☐ Personal [copy given to: 1)☐ applicant 2)☐ applicant's representative]

Exhibit shown or demonstration conducted: d)☐ Yes e)☒ No.
If Yes, brief description: _____.

Claim(s) discussed: 1, 2, 17, and 24-28.

Identification of prior art discussed: Zhang et al. (1997).

Agreement with respect to the claims f)☒ was reached. g)☐ was not reached. h)☐ N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: Proposed claim amendments were discussed to clarify the claims and to better recite the esential elements of the claimed invention. Attachments further describe these discussion conducted both by telephone and email initiated by Applicant's Representative.

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN A NON-EXTENDABLE PERIOD OF THE LONGER OF ONE MONTH OR THIRTY DAYS FROM THIS INTERVIEW DATE, OR THE MAILING DATE OF THIS INTERVIEW SUMMARY FORM, WHICHEVER IS LATER, TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached sheet.

ATTACHMENT 1

Staples, Mark

From: Sandy Livnat [slivnat@verizon.net]
Sent: Thursday, June 04, 2009 8:00 AM
To: Staples, Mark
Subject: 10/522,405 Prior to your "allowance conf" with SPE

Mark:

I'm conferencing with clients right now and we'll have proposed amended claim 1 to you within an hour or so

Sandy

Sandy Livnat, Ph.D.
Browdy and Neimark PLLC
624 Ninth Street, NW, Suite 300
Washington, DC 20001-5303
Tel: 202-628--5197
Fax: 202 -737-3528
Email: slivnat@browdyneimark.com
(General email box: mail@browdyneimark.com)
CELL: 301-807-2803
Home: 301-588-0004

Attachment 2

Staples, Mark

From: Sandy Livnat [slivnat@verizon.net]
Sent: Thursday, June 04, 2009 9:13 AM
To: Staples, Mark
Cc: Sandy (office); Livnat Home
Subject: RE: 10/522,405 Prior to your "allowance conf" with SPE
Importance: High
Attachments: Proposed new amendments for Examiner 6-4-2009.pdf

Dear Examiner Staples:

Further to our discussion yesterday, I present to you proposed amendments to claim 1 (and parallel amendments to claim24), along with some minor changes in other dependent claims to maintain consistency. Please note the footnote on page 1 as regards the appearance markings of additions/deletions, informal use of bolding and highlighting and unofficial "claim identifers".

One of the main improvements is that we now refer to the "test sample" (preamble, *etc.*) vs. "control samples" introduced later in the claim.

As discussed, the claim now provides an "X axis" value for the determinations and ends with a clearer reference back to the preamble.

Other amendments re-order the way in which certain molecules or process steps are introduced in claim 1 that should make it simpler and clearer to follow.

We wish to re-emphasize that the novelty and non-obviousness of this invention lies in large part in the fact that NucSeqI' and NucSeqII' (and any additional standards that might be used, *e.g.*, a NucSeqIII', a NucSeqIV', *etc. – see various later claims)* in the control sample are all localized on a single vector in which their ratio is known [See claim 1(2)(d)(i)]

The reason that relative CN is determined in claim 1 (rather than ending with the individual determinations in Claim 1(4) – as we touched upon yesterday - is that one distinguishing feature of this method is its improved accuracy over the prior art. Determining the ratio of NucSeqI to NucSeqII gets at that accuracy. Doing this extra step of division exploits the advantage of having the NucSeqI' and NucSeqII' on a single vector and in a known ratio to one another. Otherwise that would not have mattered. This provides the present invention with its improved accuracy as compared to using only the "concentrations" or "quantities" or "copy numbers" that are obtained in step (4).

Moreover, if it is known that the NucSeqII is always present in the starting material as, *e.g.*, 2 copies per cell, then the absolute CN of NucSeqI can be calculated from the relative CN (see claim 2). Note that way in which the "absolute" and "relative" CN are now set out in the claims is a markedl improvement over the original claim set.

If this language (or something akin to it) is found acceptable, I suggest that it would be easier for you if we submitted a supplemental amendment rather than you doing all the work entailed in cranking out an Examiner's Amendment.

IF YOU RESPOND BY EMAIL, please use both my email addresses shown in the cc box.

Thank you.

Sandy Livnat

Sandy Livnat, Ph.D.
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624 Ninth Street, NW, Suite 300
Washington, DC 20001-5303
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(General email box: mail@browdyneimark.com)
Cell: 301-807-2803

PROPOSED NEW AMENDMENTS[1]
(2009-June -04)

1. ***(proposed amended)*** A method of determining the relative copy number (CN) of a first nucleotide sequence I (NucSeqI) in a test sample using an amplification technique, said method comprising the steps of:

(1) adding to the test sample that comprises NucSeqI and a chromosome-derived second nucleotide sequence II (NucSeqII), the following ingredients:

- nucleotides,
- primers.
- polymerase
- a first probe specific ~~directed~~ to NucSeqI ~~and NucSeqI'~~, comprising a first fluorophore and a quencher, and/or ~~and optionally: any additional reagents required for amplification.wherein the sample comprises a chromosome-derived second nucleotide sequence II (NucSeqII) and~~

  a second probe specific ~~directed~~ to NucSeqII ~~and NucSeqII'~~ comprising a second fluorophore and a quencher, wherein the first fluorophore and the second fluorophore are different; and optionally
- any additional reagents required for amplification.

(2) carrying out the following amplification steps in one or more amplification cycles:

(a) amplifying NucSeqI in said test sample,

(b) amplifying NucSeqII in said test sample,

(c) in a control sample, to which said ingredients of (1) are added, amplifying at multiple dilutions a third nucleotide sequence I' (NucSeqI'), corresponding to NucSeqI ~~and present in a control sample~~**to which said first probe is also specific**, ~~at multiple dilutions~~in the presence of said first probe,

wherein the relationship of NucSeqI and NucSeqI' is defined as

---

[1] Please note that additions/deletions in the claims submitted on 5/26/09 have been incorporated (markings cleared) so that **only proposed new amendments** are shown as marked additions/deletions. Claim identifiers are "descriptive" and not intended to be "official" in this claim set. Bolding and highlighting is used "informally" to highlight certain additions that we discussed on 6/3/09

(A) NucSeqI hybridizes to the complement of NucSeqI', and
(B) NucSeqI' hybridizes to the complement of NucSeqI,
both under stringent hybridization conditions, and, if NucSeqI and NucSeqI' differ in length, the shorter of the two is at most 30% shorter than the other; and

(d) in a control sample, to which said ingredients of (1) are added, amplifying at multiple dilutions a fourth nucleotide sequence II' (NucSeqII'), corresponding to NucSeqII ~~and present in a control sample, at multiple dilutions~~**to which said second probe is also specific**, in the presence of said second probe,

wherein the relationship of NucSeqII and NucSeqII' is defined as
(A) NucSeqII hybridizes to the complement of NucSeqII', and
(B) NucSeqII' hybridizes to the complement of NucSeqII,
both under stringent hybridization conditions, and, if NucSeqII and NucSeqII' differ in length, the shorter of the two is, at most, 30% shorter than the other;

wherein

(i) NucSeqI' and NucSeqII' are both localized on a single vector in which the ratio of NucSeqI' to NucSeqII' is known,

(ii) standard curves SC_I and SC_{II} comprising at least two reference points are generated by amplification of NucSeqI' and NucSeqII', respectively, at multiple dilutions, and

(iii) at least one pair of amplification reactions (a) and (b) or (c) and (d) is performed in a single container and monitored by fluorescence during amplification;

(3) determining the results of the amplifications of step (2) expressed as threshold cycle (Ct) **as a function of quantity or concentration of the relevant amplified nucleotide sequence;**

(4) obtaining from the results in step (3) the following values:

(i) "Conc-I_{SCI}" which is the concentration or quantity in the test sample of NucSeqI determined from standard curve SC_I; and

(ii) "Conc-II_{SCII}" which is the concentration or quantity in the test sample of NucSeqII determined from standard curve SC_{II},
which standard curves express threshold cycle as a function of said concentration or quantity; and

(5) determining from the values obtained in step (4) the relative CN of NucSeqI with respect to NucSeqII by the formula:

$$\text{Relative CN} = \frac{\text{Conc-I}_{SCI}}{\text{Conc-II}_{SCII}}$$

thereby determining the relative CN **of NucSeqI in said test sample.**

2. ***(proposed amended)*** A method for determining the absolute CN of a nucleotide sequence NucSeqI in a test sample, comprising:

(a) determining the relative CN using the method of claim 18, and

(b) multiplying the relative CN by the absolute CN of NucSeqII per cell.

3. ***(amended in last resp.)*** A method according to claim 1, wherein at least two different NucSeqI' sequences, used for measuring a corresponding number of different NucSeqI sequences, are localized on a single vector.

4. *(previously presented)* A method according to claim 1 wherein the sequences of NucSeqI and NucSeqI' are the same.

5. *(previously presented)* A method according to claim 1 wherein the sequences of NucSeqII and NucSeqII' are the same.

6. ***(amended in last Resp.)*** A method according to claim 2, wherein at least two different NucSeqI' sequences, used for measuring a corresponding number of different NucSeqI, are localized on a single vector.

7. *(previously presented)* A method according to claim 2 wherein the sequences of NucSeqI and the NucSeqI' are the same.

8. *(previously presented)* A method according to claim 3 wherein the sequences of NucSeqI and the NucSeqI' are the same.

9. *(previously presented)* A method according to claim 6 wherein the sequences of NucSeqI and the NucSeqI' are the same.

10. *(previously presented)* A method according to claim 2 wherein the sequences of NucSeqII and the NucSeqII' are the same.

11. *(previously presented)* A method according to claim 3 wherein the sequences of NucSeqII and the NucSeqII' are the same.

12. *(previously presented)* A method according to claim 4 wherein the sequences of NucSeqII and the NucSeqII' are the same.

13. *(previously presented)* A method according to claim 6 wherein the sequences of NucSeqII and the NucSeqII' are the same.

14. *(previously presented)* A method according to claim 7 wherein the sequences of NucSeqII and the NucSeqII' are the same.

15. *(previously presented)* A method according to claim 8 wherein the sequences of NucSeqII and the NucSeqII' are the same.

16. *(previously presented)* A method according to claim 9 wherein the sequences of NucSeqII and the NucSeqII' are the same.

17. ***(proposed amended)*** A method according to claim 1, wherein the test sample is derived from cells.

18. *(previously presented)* A method according to claim 17, wherein an absolute CN of NucSeqII per cell is known.

19. *(previously presented)* A method according to claim 18, wherein at least two different NucSeqI' sequences used for measuring a corresponding number of different NucSeqI are localized on a single vector.

20. *(previously presented)* A method according to claim 18, wherein the sequences of NucSeqI and the NucSeqI' are the same.

21. *(previously presented)* A method according to claim 18 wherein the sequences of NucSeqII and the NucSeqII' are the same.

22. *(previously presented)* A method according to claim 19 wherein the sequences of NucSeqII and the NucSeqII' are the same.

23. *(previously presented)* A method according to claim 20 wherein the sequences of NucSeqII and the NucSeqII' are the same.

24. ***(proposed amended)*** A method of determining the relative CN of a first nucleotide sequence I (NucSeqI) in a test sample using an amplification technique, said method comprising the steps of:

(1) adding to the test sample that comprises NucSeqI and a second nucleotide sequence II (NucSeqII), the following ingredients:

- nucleotides,
- primers,
- polymerase,
- a first probe specific~~directed~~ to NucSeqI and NucSeqI', comprising a fluorophore and a quencher, ~~and optionally, any additional reagents required for amplification, wherein the sample comprises a second nucleotide sequence II (NucSeqII) and/~~or

a probe directed to NucSeqII and NucSeqII' comprising a second fluorophore and a quencher, wherein the first fluorophore and the second fluorophore are different;

- any additional reagents required for amplification,

(2) carrying out the following amplification steps in one or more amplification cycles:

(a) amplifying NucSeqI in said test sample,

(b) amplifying NucSeqII in said test sample,

(c) in a control sample, to which said ingredients of (1) are added, amplifying at multiple dilutions a third nucleotide sequence I' (NucSeqI'), corresponding to NucSeqI ~~and present in a control sample, at multiple dilutions~~ **to which said first probe is also specific**, in the presence of said first probe, wherein the relationship of NucSeqI and NucSeqI' is defined as

(A) NucSeqI hybridizes to the complement of NucSeqI', and
(B) NucSeqI' hybridizes to the complement of NucSeqI,
both under stringent hybridization conditions, and, if NucSeqI and NucSeqI' differ in length, the shorter of the two is, at most, 30% shorter than the other;

(d) in a control sample, to which said ingredients of (1) are added, amplifying at multiple dilutions a fourth nucleotide sequence II' (NucSeqII'), corresponding to NucSeqII ~~and present in a control sample, at multiple dilutions~~ **to which said second probe is also specific**, in the presence of said second probe, wherein the relationship of NucSeqII and NucSeqII' is defined as

(A) NucSeqII hybridizes to the complement of NucSeqII', and
(B) NucSeqII' hybridizes to the complement of NucSeqII,
both under stringent hybridization conditions, and, if NucSeqII and NucSeqII' differ in length, the shorter of the two is at most 30% shorter than the other;

wherein

(i) NucSeqI' and NucSeqII' are both localized on a single vector in which the ratio of NucSeqI' to NucSeqII' is known,

(ii) standard curves SC_I and SC_{II} comprising at least two reference points are generated by amplification of NucSeqI' and NucSeqII', respectively, at multiple dilutions,

(iii) at least one pair of amplification reactions (a) and (b) or (c) and (d) is performed in a single container and monitored by fluorescence during amplification, and

(3) determining the results of the amplifications of step (2) expressed as threshold cycle (Ct) as a function of quantity or concentration of the relevant amplified nucleotide sequence;

(4) obtaining from the results in step (3) the following values:

(i) "Conc-I_{SCI}" which is the concentration or quantity in the sample of NucSeqI determined from standard curve SC_I; and

(ii) "Conc-II_{SCII}" which is the concentration or quantity in the sample of NucSeqII determined from standard curve SC_{II}; and

(5) determining from the values obtained in step (4) the relative CN of NucSeqI with respect to NucSeqII by the formula:

$$\text{Relative CN} = \frac{\text{Conc-}I_{SCI}}{\text{Conc-}II_{SCII}}$$

thereby determining the relative CN of NucSeqI in said test sample.

25. *(new; **proposed amended**)* The method of claim 1 wherein the quantity in the test sample in step (4) is the number of copies of NucSeqI or NucSeqII obtained from the respective standard curves in which the quantity or relative dilution of NucSeqI' or NucSeqII', expressed as copy number, is plotted on the X-axis.

26. *(new; **proposed amended**)* The method of claim 1 wherein the concentration in the test sample in step (4) is the molar or weight concentration of NucSeqI or NucSeqII obtained from the respective standard curves in which the concentration or relative dilution of NucSeqI' or NucSeqII' is plotted on the X-axis.

27. *(new; **proposed amended**)* The method of claim 24, wherein the quantity in the test sample in step (4) is the number of copies of NucSeqI or NucSeqII obtained from the respective standard curves in which the quantity or relative dilution of NucSeqI' or NucSeqII', expressed as copy number, is plotted on the X-axis.

28. *(new; **(proposed amended)**)* The method of claim 24, wherein the concentration in the test sample in step (4) is the molar or weight concentration of NucSeqI or NucSeqII obtained from the respective standard curves in which the concentration or relative dilution of NucSeqI' or NucSeqII' is plotted on the X-axis.

ATTACHMENT 3

Staples, Mark

From: Sandy Livnat [slivnat@verizon.net]
Sent: Thursday, June 04, 2009 9:17 AM
To: Staples, Mark
Cc: Sandy (office), Livnat Home
Subject: RE: 10/522,405 Prior to your "allowance conf" with SPE
Importance: High

Oh yes.. and if you need to reach me by phone today , please use my cell phone no. 301-807-2803 as I'm not sure where I will be when.

Thanks

Sandy

From: Sandy Livnat [mailto:slivnat@verizon.net]
Sent: Thursday, June 04, 2009 9:13 AM
To: 'mark.staples@uspto.gov'
Cc: Sandy (office); Livnat Home
Subject: RE: 10/522,405 Prior to your "allowance conf" with SPE
Importance: High

Dear Examiner Staples:

Further to our discussion yesterday, I present to you proposed amendments to claim 1 (and parallel amendments to claim24), along with some minor changes in other dependent claims to maintain consistency. Please note the footnote on page 1 as regards the appearance markings of additions/deletions, informal use of bolding and highlighting and unofficial "claim identifers".

One of the main improvements is that we now refer to the "test sample" (preamble, *etc.*) vs. "control samples" introduced later in the claim.

As discussed, the claim now provides an "X axis" value for the determinations and ends with a clearer reference back to the preamble

Other amendments re-order the way in which certain molecules or process steps are introduced in claim 1 that should make it simpler and clearer to follow.

We wish to re-emphasize that the novelty and non-obviousness of this invention lies in large part in the fact that NucSeqI' and NucSeqII' (and any additional standards that might be used, *e.g.*, a NucSeqIII', a NucSeqIV', *etc. – see various later claims)* in the control sample are all localized on a single vector in which their ratio is known [See claim 1(2)(d)(i)]

The reason that relative CN is determined in claim 1 (rather than ending with the individual determinations in Claim 1(4) – as we touched upon yesterday - is that one distinguishing feature of this method is its improved accuracy over the prior art. Determining the ratio of NucSeqI to NucSeqII gets at that accuracy. Doing this extra step of division exploits the advantage of having the NucSeqI' and NucSeqII' on a single vector and in a known ratio to one another. Otherwise that would not have

mattered. This provides the present invention with its improved accuracy as compared to using only the "concentrations" or "quantities" or "copy numbers" that are obtained in step (4).

Moreover, if it is known that the NucSeqII is always present in the starting material as, *e.g.*, 2 copies per cell, then the absolute CN of NucSeqI can be calculated from the relative CN (see claim 2). Note that way in which the "absolute" and "relative" CN are now set out in the claims is a markedl improvement over the original claim set.

If this language (or something akin to it) is found acceptable, I suggest that it would be easier for you if we submitted a supplemental amendment rather than you doing all the work entailed in cranking out an Examiner's Amendment.

IF YOU RESPOND BY EMAIL, please use both my email addresses shown in the cc box.

Thank you.

Sandy Livnat

Sandy Livnat, Ph.D.
Browdy and Neimark PLLC
624 Ninth Street, NW, Suite 300
Washington, DC 20001-5303
Tel: 202-628--5197
Fax: 202-393-1012
Email: slivnat@browdyneimark.com
(General email box: mail@browdyneimark.com)
Cell: 301-807-2803

ATTACHMENT 4

Staples, Mark

From: Sandy Livnat [slivnat@verizon.net]
Sent: Friday, June 05, 2009 8:29 AM
To: Staples, Mark
Cc: Sandy (office); Mary Anne Kornbau
Subject: RE: 10/522,405 Question

Mark:

I just finished conferring with clients. I need to talk to you about one issue in the about-to-be-amended claim language.

Since it doesn't appear to make sense to start "exchanging paper", it would best be handled in a brief phone call.

I can phone you now.... would this be an OK time? Or if you prefer to phone me, I'm currently at 301-588-0004

Thanks

Sandy

Sandy Livnat, Ph.D.
Browdy and Neimark PLLC
624 Ninth Street, NW, Suite 300
Washington, DC 20001-5303
Tel: 202-628--5197
Fax: 202-393-1012
Email: slivnat@browdyneimark.com
(General email box: mail@browdyneimark.com)
CELL: 301-807-2803
Home: 301-588-0004

ATTACHMENT 5

Staples, Mark

From: Sandy Livnat [slivnat@verizon.net]
Sent: Friday, June 05, 2009 10:50 AM
To: Staples, Mark
Cc: Sandy (office); Mary Anne Kornbau
Subject: 10/522,405 PROPOSED CLAIMS and Remarks re: Support (Our Ref: Cossarizza-1)
Importance: High
Attachments: Claims - Proposed Amds (and support) for entry by Examiner's Amd (2009-06-05).doc

Mark:

Attached please find the proposed amendments we discussed and additional remarks regarding support for them.

As I understand it, this proposed claim set will be attached to an interview summary you write up, and will be "converted" into an examiner's amendment that enters the claims into the record prior to allowance.

Please contact me with any further questions or comments.

Thank you

Sandy

Sandy Livnat, Ph.D.
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Washington, DC 20001-5303
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Email: slivnat@browdyneimark.com
(General email box: mail@browdyneimark.com)
CELL: 301-807-2803
Home: 301-588-0004

PROPOSED NEW AMENDMENTS[1]
(2009-June-05)

1. *(currently amended)* A method of determining the relative copy number (CN) of a first nucleotide sequence I (NucSeqI) in a test sample using an amplification technique, said method comprising the steps of:

(1) adding to the test sample that comprises NucSeqI and a chromosome-derived second nucleotide sequence II (NucSeqII), the following ingredients:

- nucleotides,
- primers,
- polymerase
- a first probe specific to NucSeqI, comprising a first fluorophore and a quencher, and/or a second probe specific to NucSeqII comprising a second fluorophore and a quencher, wherein the first fluorophore and the second fluorophore are different; and optionally
- any additional reagents required for amplification,

(2) carrying out the following amplification steps in one or more amplification cycles:

(a) amplifying NucSeqI in said test sample,

(b) amplifying NucSeqII in said test sample,

(c) in a control sample, to which said ingredients of (1) are added, amplifying at multiple dilutions a third nucleotide sequence I' (NucSeqI') corresponding to NucSeqI to which said first probe is also specific, in the presence of said first probe,

wherein the relationship of NucSeqI and NucSeqI' is defined as

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Deleted: and optionally any additional reagents required for amplification, wherein the sample comprises a chromosome-derived second nucleotide sequence II (NucSeqII) and

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[1] Marking of claim amendments and use of claim identifiers are relative to the amended claims submitted in the Response of 5/26/2009, which, according to the Examiner, will be entered first. Therefore, all markings from those earlier filed claims have been removed - and those earlier claims are all considered to be "previously presented".

(A) NucSeqI hybridizes to the complement of NucSeqI', and

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(B) NucSeqI' hybridizes to the complement of NucSeqI,

both under stringent hybridization conditions, and, if NucSeqI and NucSeqI' differ in length, the shorter of the two is at most 30% shorter than the other; and

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(d) in a control sample, to which said ingredients of (1) are added, amplifying at multiple dilutions a fourth nucleotide sequence II' (NucSeqII') corresponding to NucSeqII to which said second probe is also specific, in the presence of said second probe,

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wherein the relationship of NucSeqII and NucSeqII' is defined as

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(A) NucSeqII hybridizes to the complement of NucSeqII', and

(B) NucSeqII' hybridizes to the complement of NucSeqII,

both under stringent hybridization conditions, and, if NucSeqII and NucSeqII' differ in length, the shorter of the two is, at most, 30% shorter than the other;

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wherein

(i) NucSeqI' and NucSeqII' are both localized on a single vector in which the ratio of NucSeqI' to NucSeqII' is known,

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(ii) standard curves SC_I and SC_{II} comprising at least two reference points are generated by amplification of NucSeqI' and NucSeqII', respectively, at multiple dilutions, wherein the starting quantity, concentration or dilution of NucSeqI' and NucSeqII' is known, and

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(iii) at least one pair of amplification reactions (a) and (b) or (c) and (d) is performed in a single container and monitored by fluorescence during amplification;

(3) determining the results of the amplifications of step (2) expressed as threshold cycle (Ct) as a function of said starting quantity, concentration or dilution;

(4) obtaining from the results in step (3) the following values:

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(i) "Conc-I_{SCI}" which is the concentration, [[or]] quantity or dilution in the test sample of NucSeqI determined from standard curve SC_I; and

(ii) "Conc-II_{SCII}" which is the concentration, [[or]] quantity or dilution in the test sample of NucSeqII determined from standard curve SC_{II},

which standard curves express threshold cycle as a function of said starting concentration, [[or]] quantity or dilution; and

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(5) determining from the values obtained in step (4) the relative CN of NucSeqI with respect to NucSeqII by the formula:

$$\text{Relative CN} = \frac{\text{Conc-}I_{SCI}}{\text{Conc-}II_{SCII}}$$

thereby determining the relative CN of NucSeqI in said test sample.

2. *(currently amended)* A method for determining the absolute CN of a nucleotide sequence NucSeqI in a test sample, comprising:

(a) determining the relative CN using the method of claim 18, and

(b) multiplying the relative CN by the absolute CN of NucSeqII per cell.

3. *(previously presented)* A method according to claim 1, wherein at least two different NucSeqI' sequences, used for measuring a corresponding number of different NucSeqI sequences, are localized on a single vector.

4. *(previously presented)* A method according to claim 1 wherein the sequences of NucSeqI and NucSeqI' are the same.

5. *(previously presented)* A method according to claim 1 wherein the sequences of NucSeqII and NucSeqII' are the same.

6. *(previously presented)* A method according to claim 2, wherein at least two different NucSeqI' sequences, used for measuring a corresponding number of different NucSeqI, are localized on a single vector.

7. *(previously presented)* A method according to claim 2 wherein the sequences of NucSeqI and the NucSeqI' are the same.

8. *(previously presented)* A method according to claim 3 wherein the sequences of NucSeqI and the NucSeqI' are the same.

9. *(previously presented)* A method according to claim 6 wherein the sequences of NucSeqI and the NucSeqI' are the same.

10. *(previously presented)* A method according to claim 2 wherein the sequences of NucSeqII and the NucSeqII' are the same.

11. *(previously presented)* A method according to claim 3 wherein the sequences of NucSeqII and the NucSeqII' are the same.

12. *(previously presented)* A method according to claim 4 wherein the sequences of NucSeqII and the NucSeqII' are the same.

13. *(previously presented)* A method according to claim 6 wherein the sequences of NucSeqII and the NucSeqII' are the same.

14. *(previously presented)* A method according to claim 7 wherein the sequences of NucSeqII and the NucSeqII' are the same.

15. *(previously presented)* A method according to claim 8 wherein the sequences of NucSeqII and the NucSeqII' are the same.

16. *(previously presented)* A method according to claim 9 wherein the sequences of NucSeqII and the NucSeqII' are the same.

17. ***(currently amended)*** A method according to claim 1, wherein the test sample is derived from cells.

18. *(previously presented)* A method according to claim 17, wherein an absolute CN of NucSeqII per cell is known.

19. *(previously presented)* A method according to claim 18, wherein at least two different NucSeqI' sequences used for measuring a corresponding number of different NucSeqI are localized on a single vector.

20. *(previously presented)* A method according to claim 18, wherein the sequences of NucSeqI and the NucSeqI' are the same.

21. *(previously presented)* A method according to claim 18 wherein the sequences of NucSeqII and the NucSeqII' are the same.

22. *(previously presented)* A method according to claim 19 wherein the sequences of NucSeqII and the NucSeqII' are the same.

23. *(previously presented)* A method according to claim 20 wherein the sequences of NucSeqII and the NucSeqII' are the same.

24. ***(currently amended)*** A method of determining the relative CN of a first nucleotide sequence I (NucSeqI) in a test sample using an amplification technique, said method comprising the steps of:

(1) adding to the test sample that comprises NucSeqI and a second nucleotide sequence II (NucSeqII), the following ingredients:

- nucleotides,
- primers,
- polymerase
- a first probe specific to NucSeqI , comprising a first fluorophore and a quencher, and/or a second probe specific to NucSeqII comprising a second fluorophore and a quencher, wherein the first fluorophore and the second fluorophore are different; and optionally
- any additional reagents required for amplification,

(2) carrying out the following amplification steps in one or more amplification cycles:

(a) amplifying NucSeqI in said test sample,

(b) amplifying NucSeqII in said test sample,

(c) in a control sample, to which said ingredients of (1) are added, amplifying at multiple dilutions a third nucleotide sequence I' (NucSeqI') corresponding to NucSeqI to which said first probe is also specific, in the presence of said first probe,

wherein the relationship of NucSeqI and NucSeqI' is defined as

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Deleted: and optionally any additional reagents required for amplification, wherein the sample comprises a chromosome-derived second nucleotide sequence II (NucSeqII) and

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(A) NucSeqI hybridizes to the complement of NucSeqI`, and

(B) NucSeqI' hybridizes to the complement of NucSeqI,

both under stringent hybridization conditions, and, if NucSeqI and NucSeqI' differ in length, the shorter of the two is at most 30% shorter than the other; and

(d) in a control sample, to which said ingredients of (1) are added, amplifying at multiple dilutions a fourth nucleotide sequence II' (NucSeqII') corresponding to NucSeqII to which said second probe is also specific, in the presence of said second probe,

wherein the relationship of NucSeqII and NucSeqII' is defined as

(A) NucSeqII hybridizes to the complement of NucSeqII`, and

(B) NucSeqII` hybridizes to the complement of NucSeqII,

both under stringent hybridization conditions, and, if NucSeqII and NucSeqII' differ in length, the shorter of the two is, at most, 30% shorter than the other;

wherein

(i) NucSeqI` and NucSeqII' are both localized on a single vector in which the ratio of NucSeqI' to NucSeqII' is known,

(ii) standard curves SC_I and SC_{II} comprising at least two reference points are generated by amplification of NucSeqI' and NucSeqII`, respectively, at multiple dilutions, wherein the starting quantity, concentration or dilution of NucSeqI` and NucSeqII` is known, and

(iii) at least one pair of amplification reactions (a) and (b) or (c) and (d) is performed in a single container and monitored by fluorescence during amplification;

(3) determining the results of the amplifications of step (2) expressed as threshold cycle (Ct) as a function of said starting quantity, concentration or dilution;

(4) obtaining from the results in step (3) the following values:

(i) "Conc-I_{SCI}" which is the concentration, [[or]] quantity or dilution in the test sample of NucSeqI determined from standard curve SC_I; and

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(ii) "Conc-II_{SCII}" which is the concentration, [[or]] quantity or dilution in the test sample of NucSeqII determined from standard curve SC_{II},

which standard curves express threshold cycle as a function of said starting concentration, [[or]] quantity or dilution; and

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(5) determining from the values obtained in step (4) the relative CN of NucSeqI with respect to NucSeqII by the formula:

$$\text{Relative CN} = \frac{\text{Conc-}I_{SCI}}{\text{Conc-}II_{SCII}}$$

thereby determining the relative CN of NucSeqI in said test sample.

25. *(currently amended)* The method of claim 1 wherein the quantity in the test sample in step (4) is the number of copies of NucSeqI or NucSeqII obtained from the respective standard curves in which the quantity or relative dilution of NucSeqI' or NucSeqII', expressed as copy number, is plotted on the X-axis.

26. *(currently amended)* The method of claim 1 wherein the concentration in the test sample in step (4) is the molar or weight concentration of NucSeqI or NucSeqII obtained from the respective standard curves in which the concentration or relative dilution of NucSeqI' or NucSeqII' is plotted on the X-axis.

27. *(currently amended)* The method of claim 24, wherein the quantity in the test sample in step (4) is the number of copies of NucSeqI or NucSeqII obtained from the respective standard curves in which the quantity or relative dilution of NucSeqI' or NucSeqII', expressed as copy number, is plotted on the X-axis.

28. *(currently amended)* The method of claim 24, wherein the concentration in the test sample in step (4) is the molar or weight concentration of NucSeqI or NucSeqII obtained from the respective standard curves in which the concentration or relative dilution of NucSeqI' or NucSeqII' is plotted on the X-axis.

REMARKS FOR PROPOSED NEW AMENDMENTS

As discussed with the Examiner by telephone interview on June 3, 4 and 5, 2009, Applicants have made the above amendments which, upon entry via Examiner's Amendment, are believed to place the case in condition for allowance. Several of the amendments are discussed in more detail below. Amendments to claim 1 have been introduced in parallel into claim 24 (with the one difference that in claim 1, NucSeqII is chromosome-derived). Many of the amendments involved rearrangement of subject matter present in the original (and earlier) claims and are thereby supported. Therefore, no new matter has been added by any of the presently proposed amendments.

Starting, Quantity Concentration or Dilution

At a number of places in claim 1, Applicants have indicated that the X axis of the standard curves is the "starting" quantity, concentration or dilution. The corresponding values for the unknowns are obtained by interpolation of these curves on the way to determining relative CN of NucSeqI. Support in the specification for the amended language is as follows (emphasis added):

Page 1, lines 15-29

c) a third nucleotide sequence I'... present in a **control sample** is amplified at **various dilutions**, and

d) a fourth nucleotide sequence II'... present in a **control sample** is amplified at **various dilutions**,

where the ratio of the **concentrations** of nucleotide sequence I' and II' is known
where the amplifications of ... nucleotide sequences I' and II' at **various dilutions** allows standard curves ... to be made, the **concentrations** of I and II are determined by using the respective standard curve SC_i, and the **relative concentrations** allows the relative copy number CN of sequence I (versus nucleotide sequence II) to be determined ...

Page 4, lines 24-26

It is also very easy to determine the DNA **concentration** and hence the **copy number** of the nucleotide sequence **per volume.**

Page 9, lines 20-21:

The standard curves were made by introducing a **known number** of **copies** of vector **per well.**

Page 9, lines 2-3:

The absolute **concentration** of the **controls** was done using limiting **dilution** assays"

Figures 1-4 – labeling of X-axis

For each figure, the X axis is labeled "Log **Starting Quantity**, copy number"

<u>**"Test" sample**</u>

Support for use of "test" sample in claim 1 and various dependent claims is present in multiple locations throughout the specification.

<u>**"Control sample"**</u>

Support for "control sample" or "control" in claim 1(2)(c) and 1(2)(d) is found at least in the cites from page 1 and 9 presented above.

Staples, Mark ATTACHMENT 6

From: Sandy Livnat [slivnat@verizon.net]
Sent: Friday, June 05, 2009 1:07 PM
To: Staples, Mark
Cc: Mary Anne Kornbau; Sandy (office)
Subject: RE: 10/522,405 PROPOSED CLAIMS and Remarks re: Support (Our Ref: Cossarizza-1)
Attachments: Claims - Proposed Amds (and support) for entry by Examiner's Amd (2009-06-05).pdf

Mark:

Fret not! The sidebar annotations, colors, etc. can be "disappeared" by resetting the "review functions" of Track Changes in MS Word. **This is how we do it for all amendments. [the only thing not affected are the double brackets which are OK appearance-wise]**

1. Open the **Track Change Bar** by right clicking in your tool bar/menu bar area on top.

2. Checkmark "Reviewing" in the box that opens - this will open the Reviewing Tool Bar.

3. Left click the drop-down arrow next to "Show" and select Options, which opens up a dialog box: **make the following settings**

"Underline color" --- "Automatic"
"Strikethrough color"---"Automatic"
"Formatting" --- "None"
"Changed lines" --- "None"
("Comments color" - irrelevant as they're not showing)
"Use Balloons" --NEVER

It should now look exactly like what you seek.

I've converted mine to pdf and attached it here too - should look exactly like the Word version on my screen (and your screen with the above settings). Or you can simply print out the pdf version for phsyical attachment to an Examiner's amendment (or for saving on your system).

We use this approach rather than "hard" underscoring" and "hard" strikethrough" because by **"accepting all changes"** you now have clean amended claims (other than manual cleanup of bracketed stuff) ready to work on for a subsequent amendment.....

Let me know how these two options work. Otherwise, if you must have a Word version with "hard" underscoring and strikethroughs,. it will take a secretaray a few hours to "get to it" and to "do it".

Thanks
Sandy

From: Staples, Mark [mailto:Mark.Staples@USPTO.GOV]
Sent: Friday, June 05, 2009 12:50 PM
To: Sandy Livnat
Subject: RE: 10/522,405 PROPOSED CLAIMS and Remarks re: Support (Our Ref: Cossarizza-1)

Please send me a copy of the amended claims in a MS Word document all in black font and without any sidebar annotations. Please keep the underlining and strikethroughs as appropriate for claim amendments.

Thank you,

Mark Staples
Patent Examiner
United States Patent and Trademark Office
Art Unit 1637
Patent Hoteling Program
Mail Stop Remsen 2D18
(571) 272-9053

From: Sandy Livnat [mailto:slivnat@verizon.net]
Sent: Friday, June 05, 2009 10:50 AM
To: Staples, Mark
Cc: Sandy (office); Mary Anne Kornbau
Subject: 10/522,405 PROPOSED CLAIMS and Remarks re: Support (Our Ref: Cossarizza-1)
Importance: High

Mark:

Attached please find the proposed amendments we discussed and additional remarks regarding support for them.

As I understand it, this proposed claim set will be attached to an interview summary you write up, and will be "converted" into an examiner's amendment that enters the claims into the record prior to allowance.

Please contact me with any further questions or comments.

Thank you

Sandy

Sandy Livnat, Ph.D.
Browdy and Neimark PLLC
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Washington, DC 20001-5303
Tel: 202-628--5197
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(General email box: mail@browdyneimark.com)
CELL: 301-807-2803
Home: 301-588-0004

PROPOSED NEW AMENDMENTS[1]
(2009-June -05)

1. *(currently amended)* A method of determining the relative copy number (CN) of a first nucleotide sequence I (NucSeqI) in a test sample using an amplification technique, said method comprising the steps of:

(1) adding to the test sample that comprises NucSeqI and a chromosome-derived second nucleotide sequence II (NucSeqII), the following ingredients:

- nucleotides,
- primers,
- polymerase
- a first probe specific ~~directed~~ to NucSeqI ~~and NucSeqI'~~, comprising a first fluorophore and a quencher, and/or ~~and optionally any additional reagents required for amplification, wherein the sample comprises a chromosome-derived second nucleotide sequence II (NucSeqII) and~~

  a second probe specific ~~directed~~ to NucSeqII ~~and NucSeqII'~~ comprising a second fluorophore and a quencher, wherein the first fluorophore and the second fluorophore are different; and optionally
- any additional reagents required for amplification.

(2) carrying out the following amplification steps in one or more amplification cycles:

(a) amplifying NucSeqI in said test sample,

(b) amplifying NucSeqII in said test sample,

(c) in a control sample, to which said ingredients of (1) are added, amplifying at multiple dilutions a third nucleotide sequence I' (NucSeqI') corresponding to NucSeqI ~~and present in a control sample~~to which said first probe is also specific, ~~at multiple dilutions~~in the presence of said first probe,

wherein the relationship of NucSeqI and NucSeqI' is defined as

---

[1] Marking of claim amendments and use of claim identifiers are **relative to** the amended claims submitted in the Response of 5/26/2009, which, according to the Examiner, will be entered first. Therefore, all markings from those earlier filed claims have been removed - and those earlier claims are all considered to be "previously presented".

(A) NucSeqI hybridizes to the complement of NucSeqI`, and

(B) NucSeqI` hybridizes to the complement of NucSeqI,

both under stringent hybridization conditions, and, if NucSeqI and NucSeqI` differ in length, the shorter of the two is at most 30% shorter than the other; and

(d) in a control sample, to which said ingredients of (1) are added, amplifying at multiple dilutions a fourth nucleotide sequence II` (NucSeqII`) corresponding to NucSeqII ~~and present in a control sample, at multiple dilutions~~to which said second probe is also specific, in the presence of said second probe,

wherein the relationship of NucSeqII and NucSeqII` is defined as

(A) NucSeqII hybridizes to the complement of NucSeqII`, and

(B) NucSeqII` hybridizes to the complement of NucSeqII,

both under stringent hybridization conditions, and, if NucSeqII and NucSeqII` differ in length, the shorter of the two is, at most, 30% shorter than the other;

wherein

(i) NucSeqI` and NucSeqII` are both localized on a single vector in which the ratio of NucSeqI` to NucSeqII` is known,

(ii) standard curves SC_I and SC_{II} comprising at least two reference points are generated by amplification of NucSeqI` and NucSeqII`, respectively, at multiple dilutions, wherein the starting quantity, concentration or dilution of NucSeqI` and NucSeqII` is known, and

(iii) at least one pair of amplification reactions (a) and (b) or (c) and (d) is performed in a single container and monitored by fluorescence during amplification;

(3) determining the results of the amplifications of step (2) expressed as threshold cycle (Ct) as a function of said starting quantity, concentration or dilution;

(4) obtaining from the results in step (3) the following values:

(i) "Conc-I_{SCI}" which is the concentration, [[or]] quantity or dilution in the test sample of NucSeqI determined from standard curve SC_I; and

(ii) "Conc-II$_{SCII}$" which is the concentration, [[or]] quantity or dilution in the test sample of NucSeqII determined from standard curve SC_{II},

which standard curves express threshold cycle as a function of said starting concentration, [[or]] quantity or dilution; and

(5) determining from the values obtained in step (4) the relative CN of NucSeqI with respect to NucSeqII by the formula:

$$\text{Relative CN} = \frac{\text{Conc-I}_{SCI}}{\text{Conc-II}_{SCII}}$$

thereby determining the relative CN of NucSeqI in said test sample.

2. ***(currently** amended)* A method for determining the absolute CN of a nucleotide sequence NucSeqI in a test sample, comprising:

(a) determining the relative CN using the method of claim 18, and

(b) multiplying the relative CN by the absolute CN of NucSeqII per cell.

3. *(previously presented)* A method according to claim 1, wherein at least two different NucSeqI' sequences, used for measuring a corresponding number of different NucSeqI sequences, are localized on a single vector.

4. *(previously presented)* A method according to claim 1 wherein the sequences of NucSeqI and NucSeqI' are the same.

5. *(previously presented)* A method according to claim 1 wherein the sequences of NucSeqII and NucSeqII' are the same.

6. *(previously presented)* A method according to claim 2, wherein at least two different NucSeqI' sequences, used for measuring a corresponding number of different NucSeqI, are localized on a single vector.

7. *(previously presented)* A method according to claim 2 wherein the sequences of NucSeqI and the NucSeqI' are the same.

8. *(previously presented)* A method according to claim 3 wherein the sequences of NucSeqI and the NucSeqI' are the same.

9. *(previously presented)* A method according to claim 6 wherein the sequences of NucSeqI and the NucSeqI' are the same.

10. *(previously presented)* A method according to claim 2 wherein the sequences of NucSeqII and the NucSeqII' are the same.

11. *(previously presented)* A method according to claim 3 wherein the sequences of NucSeqII and the NucSeqII' are the same.

12. *(previously presented)* A method according to claim 4 wherein the sequences of NucSeqII and the NucSeqII' are the same.

13. *(previously presented)* A method according to claim 6 wherein the sequences of NucSeqII and the NucSeqII' are the same.

14. *(previously presented)* A method according to claim 7 wherein the sequences of NucSeqII and the NucSeqII' are the same.

15. *(previously presented)* A method according to claim 8 wherein the sequences of NucSeqII and the NucSeqII' are the same.

16. *(previously presented)* A method according to claim 9 wherein the sequences of NucSeqII and the NucSeqII' are the same.

17. ***(currently** amended)* A method according to claim 1, wherein the test sample is derived from cells.

18. *(previously presented)* A method according to claim 17, wherein an absolute CN of NucSeqII per cell is known.

19. *(previously presented)* A method according to claim 18, wherein at least two different NucSeqI' sequences used for measuring a corresponding number of different NucSeqI are localized on a single vector.

20. *(previously presented)* A method according to claim 18, wherein the sequences of NucSeqI and the NucSeqI' are the same.

21. *(previously presented)* A method according to claim 18 wherein the sequences of NucSeqII and the NucSeqII' are the same.

22. *(previously presented)* A method according to claim 19 wherein the sequences of NucSeqII and the NucSeqII' are the same.

23. *(previously presented)* A method according to claim 20 wherein the sequences of NucSeqII and the NucSeqII' are the same.

24. ***(currently** amended)* A method of determining the relative CN of a first nucleotide sequence I (NucSeqI) in a test sample using an amplification technique, said method comprising the steps of:

(1) adding to the test sample that comprises NucSeqI and a second nucleotide sequence II (NucSeqII), the following ingredients:

- nucleotides,
- primers,
- polymerase
- a first probe specific ~~directed~~ to NucSeqI ~~and NucSeqI'~~, comprising a first fluorophore and a quencher, and/or ~~and optionally, any additional reagents required for amplification, wherein the sample comprises a chromosome-derived second nucleotide sequence II (NucSeqII) and~~
  a second probe specific ~~directed~~ to NucSeqII ~~and NucSeqII'~~ comprising a second fluorophore and a quencher, wherein the first fluorophore and the second fluorophore are different; and optionally
- any additional reagents required for amplification,

(2) carrying out the following amplification steps in one or more amplification cycles:

(a) amplifying NucSeqI in said test sample,

(b) amplifying NucSeqII in said test sample,

(c) in a control sample, to which said ingredients of (1) are added, amplifying at multiple dilutions a third nucleotide sequence I' (NucSeqI') corresponding to

NucSeqI ~~and present in a control sample~~to which said first probe is also specific, ~~at multiple dilutions~~in the presence of said first probe,

wherein the relationship of NucSeqI and NucSeqI' is defined as

(A) NucSeqI hybridizes to the complement of NucSeqI', and

(B) NucSeqI' hybridizes to the complement of NucSeqI,

both under stringent hybridization conditions, and, if NucSeqI and NucSeqI' differ in length, the shorter of the two is at most 30% shorter than the other; and

(d) in a control sample, to which said ingredients of (1) are added, amplifying at multiple dilutions a fourth nucleotide sequence II' (NucSeqII') corresponding to NucSeqII ~~and present in a control sample, at multiple dilutions~~to which said second probe is also specific, in the presence of said second probe,

wherein the relationship of NucSeqII and NucSeqII' is defined as

(A) NucSeqII hybridizes to the complement of NucSeqII', and

(B) NucSeqII' hybridizes to the complement of NucSeqII,

both under stringent hybridization conditions, and, if NucSeqII and NucSeqII' differ in length, the shorter of the two is, at most, 30% shorter than the other;

wherein

(i) NucSeqI' and NucSeqII' are both localized on a single vector in which the ratio of NucSeqI' to NucSeqII' is known,

(ii) standard curves SC_I and SC_{II} comprising at least two reference points are generated by amplification of NucSeqI' and NucSeqII', respectively, at multiple dilutions, wherein the starting quantity, concentration or dilution of NucSeqI' and NucSeqII'' is known, and

(iii) at least one pair of amplification reactions (a) and (b) or (c) and (d) is performed in a single container and monitored by fluorescence during amplification;

(3) determining the results of the amplifications of step (2) expressed as threshold cycle (Ct) as a function of said starting quantity, concentration or dilution;

(4) obtaining from the results in step (3) the following values:

(i) "Conc-I_{SCI}" which is the concentration, [[or]] quantity or dilution in the test sample of NucSeqI determined from standard curve SC_I; and

(ii) "Conc-II_{SCII}" which is the concentration, [[or]] quantity or dilution in the test sample of NucSeqII determined from standard curve SC_{II},

which standard curves express threshold cycle as a function of said starting concentration, [[or]] quantity or dilution; and

(5) determining from the values obtained in step (4) the relative CN of NucSeqI with respect to NucSeqII by the formula:

$$\text{Relative CN} = \frac{\text{Conc-I}_{SCI}}{\text{Conc-II}_{SCII}}$$

thereby determining the relative CN of NucSeqI in said test sample.

25. ***(currently amended)*** The method of claim 1 wherein the quantity in the test sample in step (4) is the number of copies of NucSeqI or NucSeqII obtained from the respective standard curves in which the quantity or relative dilution of NucSeqI' or NucSeqII', expressed as copy number, is plotted on the X-axis.

26. ***(currently amended)*** The method of claim 1 wherein the concentration in the test sample in step (4) is the molar or weight concentration of NucSeqI or NucSeqII obtained from the respective standard curves in which the concentration or relative dilution of NucSeqI' or NucSeqII' is plotted on the X-axis.

27. ***(currently amended)*** The method of claim 24, wherein the quantity in the test sample in step (4) is the number of copies of NucSeqI or NucSeqII obtained from the respective standard curves in which the quantity or relative dilution of NucSeqI' or NucSeqII', expressed as copy number, is plotted on the X-axis.

28. ***(currently amended)*** The method of claim 24, wherein the concentration in the test sample in step (4) is the molar or weight concentration of NucSeqI or NucSeqII obtained from the respective standard curves in which the concentration or relative dilution of NucSeqI' or NucSeqII' is plotted on the X-axis.

REMARKS FOR PROPOSED NEW AMENDMENTS

As discussed with the Examiner by telephone interview on June 3, 4 and 5, 2009, Applicants have made the above amendments which, upon entry via Examiner's Amendment, are believed to place the case in condition for allowance. Several of the amendments are discussed in more detail below. Amendments to claim 1 have been introduced in parallel into claim 24 (with the one difference that in claim 1, NucSeqII is chromosome-derived). Many of the amendments involved rearrangement of subject matter present in the original (and earlier) claims and are thereby supported. Therefore, no new matter has been added by any of the presently proposed amendments.

Starting, Quantity Concentration or Dilution

At a number of places in claim 1, Applicants have indicated that the X axis of the standard curves is the "starting" quantity, concentration or dilution. The corresponding values for the unknowns are obtained by interpolation of these curves on the way to determining relative CN of NucSeqI. Support in the specification for the amended language is as follows (emphasis added):

Page 1, lines 15-29

c) a third nucleotide sequence I'…present in a **control sample** is amplified at **various dilutions**, and

d) a fourth nucleotide sequence II'… present in a **control sample** is amplified at **various dilutions**,

where the ratio of the **concentrations** of nucleotide sequence I' and II' is known
where the amplifications of … nucleotide sequences I' and II' at **various dilutions** allows standard curves … to be made, the **concentrations** of I and II are determined by using the respective standard curve SC_i, and the **relative concentrations** allows the relative copy number CN of sequence I (versus nucleotide sequence II) to be determined …

Page 4, lines 24-26

It is also very easy to determine the DNA **concentration** and hence the **copy number** of the nucleotide sequence **per volume.**

Page 9, lines 20-21:

The standard curves were made by introducing a **known number** of **copies** of vector **per well.**

Page 9, lines 2-3:

The absolute **concentration** of the **controls** was done using limiting **dilution** assays"

Figures 1-4 – labeling of X-axis

For each figure, the X axis is labeled "Log **Starting Quantity**, copy number"

"Test" sample

Support for use of "test" sample in claim 1 and various dependent claims is present in multiple locations throughout the specification.

"Control sample"

Support for "control sample" or "control" in claim 1(2)(c) and 1(2)(d) is found at least in the cites from page 1 and 9 presented above.